PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 May 2000 (25.05.00)	
International application No. PCT/US99/23240	**Applicant's or agent's file reference** 4239-53372
International filing date (day/month/year) 05 October 1999 (05.10.99)	**Priority date** (day/month/year) 06 October 1998 (06.10.98)
Applicant TOSATO, Giovanna et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 April 2000 (27.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia RANAIVOJAONA
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PATENT COOPERATION TREATY

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REC'D 11 JAN 2001

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4239-53372		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23240	05/10/1999	06/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/11		
Applicant THE GOVERNMENT OF THE UNITED STATES ... et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/04/2000	09.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/23240

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-40	as originally filed

Claims, No.:

1-62	as received on	02/10/2000	with letter of	29/09/2000

Drawings, sheets:

1/27-27/27	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29, 34, 38, 42-62
	No:	Claims	30-33, 35-37, 39-41
Inventive step (IS)	Yes:	Claims	1-29, 42-49, 56-62
	No:	Claims	30-41, 50-55
Industrial applicability (IA)	Yes:	Claims	30-54, 56-59, 62
	No:	Claims	1-29, 55, 60, 61

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

I. Basis

The documents mentioned in the present International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

Sequence listing pages 1-17 are also included in the basis of the present assessment.

No specific basis has been identified for newly introduced claim 56, part (e). Hence, it appears that said claim offends against Rule 70.2(c), PCT.

II. Priority

The priority document teaches that 60 and 49aa fragments (Seq. ID Nos 4 and 5 in prio doc.) have endothelial growth inhibitory activity. Further, it is shown that calreticulin and its 180 aa N-terminal fragment are active in inhibition of angiogenesis and in tumor suppression (presumably due to angiogenesis inhibition). Unspecified active fragments are also referred to. Since the skilled person would seek active fragments within fragments having known activity, the priority claim cannot be considered to extend to fragments outside of the 180 aa N-terminal fragment (or in the case of endothelial growth inhibition to fragments outside the active 49 aa fragment).

IV. Lack of Unity

Fragments of a protein which do not share a common function cannot be considered to form a unitary group. Nevertheless, for practical reasons, no unity objection shall be persued in the International Phase.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- Novelty (Art.33(2) PCT)

D1 (and in an abreviated version D6) disclose a method of inhibiting restenosis or

atherosclerosis using calreticulin. The C-domain is responsible for the effect. Whole calreticulin or fragments of 6-100 aa of the C-terminal (producable optionally by recombinant techniques) are considered preferable for use. Restenosis, however, does not involve endothelial cell growth or angiogenesis. Rather, restenosis involves smooth muscle proliferation. D1 anticipates claims 30-33 (particularly where reference made to Seq. ID No.9), 35-37 and 39-41.

D2 and D5 disclose the modulation of hormone responsiveness using calreticulin and fragments or mimetics thereof. The peptides must include the sequence K(G/A/V)FF(K/R)R, which is found in DNA-binding domain of numerous hormone receptors. Document no longer considered relevant.

D3 discloses wound-healing applications of calreticulin. The reticulin is intended to reduce scar formation during wound healing. It is postulated to mediate this effect via stimulation of growth factor expression and collagen organization. This document is not considered of particular relevance.

D4 discloses the use of calreticulin ot prevent thrombosis (blood clotting). The C-domain is considered critical since it mediates binding to Factor IX. D4 is not considered of particular relevance.

D9 discloses expression of calreticulin N, P and C domains in E. coli. The N and P domains were shown to bind C1q and thus to have relevance to C1q-mediated inflammatory response. Anticipates claim 30 and intrinsically claims 32 and 33. Since the N domain presumably binds sequence ID Nos 10 and 11, further claims are not considered anticipated.

D10 discloses 1-24 and 7-24 aa fragments of calreticulin which were used to demonstrate autoantibodies to calreticulin in diverse autoimmune conditions. Anticipates claim 30.

- **Inventive Step (Art.33(3) PCT)**

Applicant appears to be the first to discover the N-domain activities of calreticulin underlying the present application. There is no indication in the prior art that such

activity should be sought within the calreticulin protein. Thus, all adequately limited claims can be considered inventive.

The present claims are only rarely limited to specific uses and relevantly active fragments of calreticulin and thus a meaningful examination of the inventive step of individual claims is barely possible. Only the following claims can be considered to solve the problem overcome by the underlying inventive concept: 1-29, 42-49, 56-62. This statement is provisional for all claims discussed in section VIII until the clarity objections to these claims has been adequately adressed and the claims have been limited to fragments that actually solve the problem in hand.

- **Industrial Applicability (Art.33(4) PCT)**

For the assessment of the present claims 1-29, 55, 60, 61 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 1-29, 55, 60, 61 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

Claims 1, 5, 12, 26, 39, 56, 57, 60-62 - variants not defined

At least claims 4, 8, 26, 28, 30, 32 and 56 relate in part to sequences which have been shown to be irrelevant to the activities upon which application is based. The

sequences together cover the whole of the calreticulin molecule. Only Seq. ID No. 5 in combination with a functional definition is really acceptable as a basis for broad fragment claims.

Claim 30 - fragments taken from parts of 1-180 fragment which shown not to have activity (thus irrelevant to solution) + Seq ID No.9 relates to sequence outside of N-terminal 180 aa. Clearly, the activity which the application is based on resides in the 120-180 region. The claims must be drafted accordingly.

Claims 34 and 38 relate to identifying therapeutic fragments of reticulin which do not bind particular steroid receptor sequences. The methods do not define in what manner the fragments are therapeutically effective and thus extend beyond inventive concept. Further, significance of negative criterion to functionality is not clear - also which fragments exactly excluded by this criteria is not clear.

Claims 35 and 39 are product-by-process claims - style of formulation unacceptable.

Claims 50-54 - mimetics are not allowable unless structurally and functionally defined.

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
4239-53372		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23240	05/10/1999	06/10/1998
International Patent Classification (IPC) or national classification and IPC C12N15/11		
Applicant THE GOVERNMENT OF THE UNITED STATES ... et al.		

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   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/04/2000	09.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/23240

I. Basis of the report

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Description, pages:

1-40	as originally filed				

Claims, No.:

1-62	as received on	02/10/2000	with letter of	29/09/2000	

Drawings, sheets:

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2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

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   - ☐ the language of publication of the international application (under Rule 48.3(b)).
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3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
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II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

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IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

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   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-29, 34, 38, 42-62
	No:	Claims	30-33, 35-37, 39-41
Inventive step (IS)	Yes:	Claims	1-29, 42-49, 56-62
	No:	Claims	30-41, 50-55
Industrial applicability (IA)	Yes:	Claims	30-54, 56-59, 62
	No:	Claims	1-29, 55, 60, 61

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

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I. Basis

The documents mentioned in the present International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

Sequence listing pages 1-17 are also included in the basis of the present assessment.

No specific basis has been identified for newly introduced claim 56, part (e). Hence, it appears that said claim offends against Rule 70.2(c), PCT.

II. Priority

The priority document teaches that 60 and 49aa fragments (Seq. ID Nos 4 and 5 in prio doc.) have endothelial growth inhibitory activity. Further, it is shown that calreticulin and its 180 aa N-terminal fragment are active in inhibition of angiogenesis and in tumor suppression (presumably due to angiogenesis inhibition). Unspecified active fragments are also referred to. Since the skilled person would seek active fragments within fragments having known activity, the priority claim cannot be considered to extend to fragments outside of the 180 aa N-terminal fragment (or in the case of endothelial growth inhibition to fragments outside the active 49 aa fragment).

IV. Lack of Unity

Fragments of a protein which do not share a common function cannot be considered to form a unitary group. Nevertheless, for practical reasons, no unity objection shall be persued in the International Phase.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- Novelty (Art.33(2) PCT)

D1 (and in an abreviated version D6) disclose a method of inhibiting restenosis or

atherosclerosis using calreticulin. The C-domain is responsible for the effect. Whole calreticulin or fragments of 6-100 aa of the C-terminal (producable optionally by recombinant techniques) are considered preferable for use. Restenosis, however, does not involve endothelial cell growth or angiogenesis. Rather, restenosis involves smooth muscle proliferation. D1 anticipates claims 30-33 (particularly where reference made to Seq. ID No.9), 35-37 and 39-41.

D2 and D5 disclose the modulation of hormone responsiveness using calreticulin and fragments or mimetics thereof. The peptides must include the sequence K(G/A/V)FF(K/R)R, which is found in DNA-binding domain of numerous hormone receptors. Document no longer considered relevant.

D3 discloses wound-healing applications of calreticulin. The reticulin is intended to reduce scar formation during wound healing. It is postulated to mediate this effect via stimulation of growth factor expression and collagen organization. This document is not considered of particular relevance.

D4 discloses the use of calreticulin ot prevent thrombosis (blood clotting). The C-domain is considered critical since it mediates binding to Factor IX. D4 is not considered of particular relevance.

D9 discloses expression of calreticulin N, P and C domains in E. coli. The N and P domains were shown to bind C1q and thus to have relevance to C1q-mediated inflammatory response. Anticipates claim 30 and intrinsically claims 32 and 33. Since the N domain presumably binds sequence ID Nos 10 and 11, further claims are not considered anticipated.

D10 discloses 1-24 and 7-24 aa fragments of calreticulin which were used to demonstrate autoantibodies to calreticulin in diverse autoimmune conditions. Anticipates claim 30.

- **Inventive Step (Art.33(3) PCT)**

Applicant appears to be the first to discover the N-domain activities of calreticulin underlying the present application. There is no indication in the prior art that such

activity should be sought within the calreticulin protein. Thus, all adequately limited claims can be considered inventive.

The present claims are only rarely limited to specific uses and relevantly active fragments of calreticulin and thus a meaningful examination of the inventive step of individual claims is barely possible. Only the following claims can be considered to solve the problem overcome by the underlying inventive concept: 1-29, 42-49, 56-62. This statement is provisional for all claims discussed in section VIII until the clarity objections to these claims has been adequately adressed and the claims have been limited to fragments that actually solve the problem in hand.

- **Industrial Applicability (Art.33(4) PCT)**

For the assessment of the present claims 1-29, 55, 60, 61 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Claims 1-29, 55, 60, 61 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

VIII. **Certain observations**

- **Clarity (Art.6 PCT)**

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At least claims 4, 8, 26, 28, 30, 32 and 56 relate in part to sequences which have been shown to be irrelevant to the activities upon which application is based. The

sequences together cover the whole of the calreticulin molecule. Only Seq. ID No. 5 in combination with a functional definition is really acceptable as a basis for broad fragment claims.

Claim 30 - fragments taken from parts of 1-180 fragment which shown not to have activity (thus irrelevant to solution) + Seq ID No.9 relates to sequence outside of N-terminal 180 aa. Clearly, the activity which the application is based on resides in the 120-180 region. The claims must be drafted accordingly.

Claims 34 and 38 relate to identifying therapeutic fragments of reticulin which do not bind particular steroid receptor sequences. The methods do not define in what manner the fragments are therapeutically effective and thus extend beyond inventive concept. Further, significance of negative criterion to functionality is not clear - also which fragments exactly excluded by this criteria is not clear.

Claims 35 and 39 are product-by-process claims - style of formulation unacceptable.

Claims 50-54 - mimetics are not allowable unless structurally and functionally defined.

Claims

What is claimed is:

1. A method of inhibiting endothelial cell growth, comprising contacting endothelial cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

2. The method of claim 1 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

3. The method of claim 1 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

4. The method of claim 1 wherein the therapeutically effective fragment of calreticulin consists essentially of an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 3;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 4;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(e) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

f) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

5. A method of inhibiting angiogenesis in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

6. The method of claim 5 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

7. The method of claim 5 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

8. The method of claim 5 wherein the therapeutically effective fragment of calreticulin consists essentially of an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 3;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 4;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(e) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

f) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

9. The method of claim 5 wherein the angiogenesis is associated with a disease, other than a tumor, that is associated with neovascularization.

10. The method of claim 9 wherein angiogenesis is inhibited in a disease selected from a group consisting of diabetic retinopathy, retrolental fibroplasia, trachoma, neovascular glaucoma, psoriasis, angiofibromas, immune-inflammation, atherosclerosis, excessive wound repair, retinal neovascularization, macular degeneration, corneal graft rejection, contact lens overwear, Crohn's disease and non-immune inflammation.

11. The method of claim 9 wherein the disease is selected from a group consisting of rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, Goodpasture's Syndrome, systemic vasculitis, scleroderma, Sjogren's syndrome, sarcoidosis and primary biliary cirrhosis.

12. A method of treatment of Kaposi's sarcoma, comprising administering to the subject an effective amount of pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(d) therapeutically effective variants of calreticulin; and

(e) calreticulin.

13. The method of claim 5 further comprising providing a second anti-angiogenic agent selected from a group consisting of platelet-factor-4, IP-10 (interferon (IFN)-γ inducible protein-10), MIG (Monokine induced by IFN-γ), INF-γ, IFN-α, angiostatin, endostatin, fumagillin, AGM-1470, thrombospondin, a fragment of prolactin, antibody against the integrin αvβ3, IL-12, cleaved conformation of the serpin antithrombin, thalidomide, and mixtures thereof.

14. The method of claim 5 further comprising administering a chemotherapeutic agent.

15. The method of claim 5 further comprising administering a hormone.

16. The method of claim 5 further comprising administering an anti-inflamatory agent.

17. The method of claim 5 further comprising administering an anti-viral agent.

18. The method of claim 5 wherein the angiogenesis is inhibited in pregnancy.

19. The method of claim 5 wherein the angiogenesis is inhibited to terminate pregnancy.

20. The method of claim 5 wherein the angiogenesis is inhibited in periodontal disease.

21. The method of claim 20 further comprising administering an antibiotic.

22. The method of claim 5 wherein the angiogenesis is inhibited in radiation induced injury.

23. The method of claim 5 wherein the angiogenesis is inhibited in chemotherapy induced injury.

24. The method of claim 5 wherein the pharmaceutical composition inhibits angiogenesis which is stimulated by an angiogenesis inducer selected from a group consisting of, basic fibroblast growth factor, acidic fibroblast growth factor, Vascular Endothelial Growth Factor (VEGF), hepatocyte growth factor, Interleukin (IL)-15, IL-8, platelet-derived endothelial cell growth factor (PDECGF), Transforming Growth Factor (TGF)-β, Tumor necrosis Factor (TNF)α, angiogenin, Cripto, and mixtures thereof.

25. The method of claim 5, wherein the subject is immunocompromized due to T-lymphocyte deficiency.

26. A method of inhibiting tumor growth, comprising contacting tumor cells with an effective amount of a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) the amino acid sequence shown in SEQ ID NO: 5

(b) the amino acid sequence shown in Seq. I.D.No. 6 ;

(c) the amino acid sequence shown in Seq. I.D. No. 8;

(d) the amino acid sequence shown in Seq. I.D. No. 9; and

(e) amino acid sequences consisting essentially of fragments and variants of the sequences of (a), (b), (c), and (d), wherein the amino acid sequence inhibits tumor growth.

27. The method of claim 26 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

28. The method of claim 26 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

29. The method of claim 26 wherein the tumor growth occurs in a subject and is inhibited by administering to the subject an effective amount of the pharmaceutical composition.

30. A protein consisting essentially of an amino acid sequence selected from the group consisting of:

4;

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9,

wherein the protein has a function selected from the group consisting of: angiogenesis inhibition, tumor growth inhibition, radiation induced injury inhibition, chemotherapeutic induced injury inhibition, and endothelial cell growth inhibition.

31. A composition comprising a protein according to claim 30, and a pharmaceutically acceptable carrier.

32. A vector comprising a nucleotide sequence encoding a protein with an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

33. A host cell comprising the vector according to claim 32.

34. A method of isolating a therapeutically effective fragment of calreticulin from a sample comprising:

(a) contacting the sample with at least one peptide where the peptide has the amino acid sequence selected from the group consisting of Seq. I.D. No. 10 and Seq. I.D. No. 11;

(b) recovering the portion of the sample that does not bind to the peptide; and

(c) assaying the recovered portion of the sample for therapeutic activity.

35. A therapeutically effective fragment of calreticulin identified by the method of claim 34.

36. A vector comprising a nucleotide sequence encoding the therapeutically effective fragment of claim 35.

37. A host cell comprising the vector according to claim 36.

38. A method of isolating a therapeutically effective variant of calreticulin from a sample comprising:

(a) contacting the sample with at least one peptide where the peptide has the amino acid sequence selected from the group consisting of Seq. I.D. No. 10 and Seq. I.D. No. 11;

(b) recovering the portion of the sample that does not bind to the peptide; and

(c) assaying the recovered portion of the sample for therapeutic activity.

39. A therapeutically effective variant of calreticulin identified by the method of claim 38.

40. A vector comprising a nucleotide sequence encoding the therapeutically effective variant of claim 39.

41. A host cell comprising the vector according to claim 40.

42. A therapeutically effective fragment of calreticulin that:

(a) does not bind to the amino acid sequence shown in Seq. I.D. No. 10; and

(b) displays a biological activity selected from the group consisting of: at least 30% inhibition of angiogenesis, at least 30% inhibition of tumor growth, and at least 30% inhibition of endothelial cell growth.

43. The therapeutically effective fragment of claim 42, wherein the biological activity is selected from the group consisting of: at least 40% inhibition of angiogenesis, at least 40% inhibition of tumor growth, and at least 40% inhibition of endothelial cell growth.

44. A vector comprising a nucleotide sequence encoding the therapeutically effective fragment of claim 42.

45. A host cell comprising the vector according to claim 44.

46. A therapeutically effective variant of calreticulin that:

(a) does not bind to the amino acid sequence shown in Seq. I.D. No. 10; and

(b) displays a biological activity selected from the group consisting of: at least 30% inhibition of angiogenesis, at least 30% inhibition of tumor growth, and at least 30% inhibition of endothelial cell growth.

47. The therapeutically effective fragment of claim 43, wherein the biological activity is selected from the group consisting of: at least 40% inhibition of angiogenesis, at least 40% inhibition of tumor growth, and at least 40% inhibition of endothelial cell growth.

48. A vector comprising a nucleotide sequence encoding the therapeutically effective variant of claim 47.

49. A host cell comprising the vector according to claim 48.

50. A mimetic of the protein of claim 30.

51. A mimetic of the therapeutically effective fragment of claim 35.

52. A mimetic of the therapeutically effective variant of claim 39.

53. A mimetic of the therapeutically effective fragment of claim 42.

54. A mimetic of the therapeutically effective variant of claim 46.

55. The method of claim 5 further comprising administering radiation therapy.

56. The method of any one of claims 1, or 5, wherein the therapeutically effective fragment of calreticuliln consists essentially of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence shown in SEQ ID NO: 5

(b) the amino acid sequence shown in Seq. I.D.No. 6 ;

(c) the amino acid sequence shown in Seq. I.D. No. 8;

(d) the amino acid sequence shown in Seq. I.D. No. 9; and

(e) amino acid sequences comprising fragments and variants of the sequences of (a), (b), (c), and (d), wherein the amino acid sequence inhibits tumor growth.

57. The use of a therapeutically effective fragment of the amino acid sequence shown in SEQ ID NO: 4 or a therapeutically effective variants of the amino acid sequence shown in Seq. I.D. No. 4 in the manufacture of a medicament to treat unwanted endothelial cell growth, or angiogenesis.

58. The use of a therapeutically effective fragment of claim 57, wherein the medicament manufactured is used to inhibit angiogenesis.

59. The use of a therapeutically effective fragment of claim 57, wherein the medicament manufactured is used to inhibit endothelial cell growth.

60. A method of inhibiting radiation induced injury, comprising contacting cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

61. A method of inhibiting chemotherapy induced injury, comprising contacting cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

62. The use of therapeutically effective fragments of calreticulin, variants of calreticulin, or calreticulin in the manufacture of a medicament to treat or prevent radiation induced injury or chemotherapeutic induced injury.

Claims

What is claimed is:

1. A method of inhibiting endothelial cell growth, comprising contacting endothelial cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

2. The method of claim 1 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

3. The method of claim 1 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

4. The method of claim 1 wherein the therapeutically effective fragment of calreticulin consists essentially of an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 3;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 4;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(e) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

f) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

5. A method of inhibiting angiogenesis in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

6. The method of claim 5 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

7. The method of claim 5 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

8. The method of claim 5 wherein the therapeutically effective fragment of calreticulin consists essentially of an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 3;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 4;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(e) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

f) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

9. The method of claim 5 wherein the angiogenesis is associated with a disease, other than a tumor, that is associated with neovascularization.

10. The method of claim 9 wherein angiogenesis is inhibited in a disease selected from a group consisting of diabetic retinopathy, retrolental fibroplasia, trachoma, neovascular glaucoma, psoriasis, angiofibromas, immune-inflammation, atherosclerosis, excessive wound repair, retinal neovascularization, macular degeneration, corneal graft rejection, contact lens overwear, Crohn's disease and non-immune inflammation.

11. The method of claim 9 wherein the disease is selected from a group consisting of rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, Goodpasture's Syndrome, systemic vasculitis, scleroderma, Sjogren's syndrome, sarcoidosis and primary biliary cirrhosis.

12. A method of treatment of Kaposi's sarcoma, comprising administering to the subject an effective amount of pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(d) therapeutically effective variants of calreticulin; and

(e) calreticulin.

13. The method of claim 5 further comprising providing a second anti-angiogenic agent selected from a group consisting of platelet-factor-4, IP-10 (interferon (IFN)-γ inducible protein-10), MIG (Monokine induced by IFN-γ), INF-γ, IFN-α, angiostatin, endostatin, fumagillin, AGM-1470, thrombospondin, a fragment of prolactin, antibody against the integrin αvβ3, IL-12, cleaved conformation of the serpin antithrombin, thalidomide, and mixtures thereof.

14. The method of claim 5 further comprising administering a chemotherapeutic agent.

15. The method of claim 5 further comprising administering a hormone.

16. The method of claim 5 further comprising administering an anti-inflamatory agent.

17. The method of claim 5 further comprising administering an anti-viral agent.

18. The method of claim 5 wherein the angiogenesis is inhibited in pregnancy.

19. The method of claim 5 wherein the angiogenesis is inhibited to terminate pregnancy.

20. The method of claim 5 wherein the angiogenesis is inhibited in periodontal disease.

21. The method of claim 20 further comprising administering an antibiotic.

22. The method of claim 5 wherein the angiogenesis is inhibited in radiation induced injury.

23. The method of claim 5 wherein the angiogenesis is inhibited in chemotherapy induced injury.

24. The method of claim 5 wherein the pharmaceutical composition inhibits angiogenesis which is stimulated by an angiogenesis inducer selected from a group consisting of, basic fibroblast growth factor, acidic fibroblast growth factor, Vascular Endothelial Growth Factor (VEGF), hepatocyte growth factor, Interleukin (IL)-15, IL-8, platelet-derived endothelial cell growth factor (PDECGF), Transforming Growth Factor (TGF)-β, Tumor necrosis Factor (TNF)α, angiogenin, Cripto, and mixtures thereof.

25. The method of claim 5, wherein the subject is immunocompromized due to T-lymphocyte deficiency.

26. A method of inhibiting tumor growth, comprising contacting tumor cells with an effective amount of a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) the amino acid sequence shown in SEQ ID NO: 5

(b) the amino acid sequence shown in Seq. I.D.No. 6 ;

(c) the amino acid sequence shown in Seq. I.D. No. 8;

(d) the amino acid sequence shown in Seq. I.D. No. 9; and

(e) amino acid sequences consisting essentially of fragments and variants of the sequences of (a), (b), (c), and (d), wherein the amino acid sequence inhibits tumor growth.

27. The method of claim 26 wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

28. The method of claim 26 wherein the therapeutically effective fragment of calreticulin comprises a polypeptide of about 180 amino acids from the N-terminus of calreticulin.

29. The method of claim 26 wherein the tumor growth occurs in a subject and is inhibited by administering to the subject an effective amount of the pharmaceutical composition.

30. A protein consisting essentially of an amino acid sequence selected from the group consisting of:

4;

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9,

wherein the protein has a function selected from the group consisting of: angiogenesis inhibition, tumor growth inhibition, radiation induced injury inhibition, chemotherapeutic induced injury inhibition, and endothelial cell growth inhibition.

31. A composition comprising a protein according to claim 30, and a pharmaceutically acceptable carrier.

32. A vector comprising a nucleotide sequence encoding a protein with an amino acid sequence selected from the group consisting of:

(a) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 5;

(b) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 6;

(c) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 8; and

(d) at least 5 contiguous amino acid residues of the sequence shown in Seq. I.D. No. 9.

33. A host cell comprising the vector according to claim 32.

34. A method of isolating a therapeutically effective fragment of calreticulin from a sample comprising:

(a) contacting the sample with at least one peptide where the peptide has the amino acid sequence selected from the group consisting of Seq. I.D. No. 10 and Seq. I.D. No. 11;

(b) recovering the portion of the sample that does not bind to the peptide; and

(c) assaying the recovered portion of the sample for therapeutic activity.

35. A therapeutically effective fragment of calreticulin identified by the method of claim 34.

36. A vector comprising a nucleotide sequence encoding the therapeutically effective fragment of claim 35.

37. A host cell comprising the vector according to claim 36.

38. A method of isolating a therapeutically effective variant of calreticulin from a sample comprising:

(a) contacting the sample with at least one peptide where the peptide has the amino acid sequence selected from the group consisting of Seq. I.D. No. 10 and Seq. I.D. No. 11;

(b) recovering the portion of the sample that does not bind to the peptide; and

(c) assaying the recovered portion of the sample for therapeutic activity.

39. A therapeutically effective variant of calreticulin identified by the method of claim 38.

40. A vector comprising a nucleotide sequence encoding the therapeutically effective variant of claim 39.

41. A host cell comprising the vector according to claim 40.

42. A therapeutically effective fragment of calreticulin that:

(a) does not bind to the amino acid sequence shown in Seq. I.D. No. 10; and

(b) displays a biological activity selected from the group consisting of: at least 30% inhibition of angiogenesis, at least 30% inhibition of tumor growth, and at least 30% inhibition of endothelial cell growth.

43. The therapeutically effective fragment of claim 42, wherein the biological activity is selected from the group consisting of: at least 40% inhibition of angiogenesis, at least 40% inhibition of tumor growth, and at least 40% inhibition of endothelial cell growth.

44. A vector comprising a nucleotide sequence encoding the therapeutically effective fragment of claim 42.

45. A host cell comprising the vector according to claim 44.

46. A therapeutically effective variant of calreticulin that:

(a) does not bind to the amino acid sequence shown in Seq. I.D. No. 10; and

(b) displays a biological activity selected from the group consisting of: at least 30% inhibition of angiogenesis, at least 30% inhibition of tumor growth, and at least 30% inhibition of endothelial cell growth.

47. The therapeutically effective fragment of claim 43, wherein the biological activity is selected from the group consisting of: at least 40% inhibition of angiogenesis, at least 40% inhibition of tumor growth, and at least 40% inhibition of endothelial cell growth.

48. A vector comprising a nucleotide sequence encoding the therapeutically effective variant of claim 47.

49. A host cell comprising the vector according to claim 48.

50. A mimetic of the protein of claim 30.

51. A mimetic of the therapeutically effective fragment of claim 35.

52. A mimetic of the therapeutically effective variant of claim 39.

53. A mimetic of the therapeutically effective fragment of claim 42.

54. A mimetic of the therapeutically effective variant of claim 46.

55. The method of claim 5 further comprising administering radiation therapy.

56. The method of any one of claims 1, or 5, wherein the therapeutically effective fragment of calreticuliln consists essentially of an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence shown in SEQ ID NO: 5

(b) the amino acid sequence shown in Seq. I.D.No. 6 ;

(c) the amino acid sequence shown in Seq. I.D. No. 8;

(d) the amino acid sequence shown in Seq. I.D. No. 9; and

(e) amino acid sequences comprising fragments and variants of the sequences of (a), (b), (c), and (d), wherein the amino acid sequence inhibits tumor growth.

57. The use of a therapeutically effective fragment of the amino acid sequence shown in SEQ ID NO: 4 or a therapeutically effective variants of the amino acid sequence shown in Seq. I.D. No. 4 in the manufacture of a medicament to treat unwanted endothelial cell growth, or angiogenesis.

58. The use of a therapeutically effective fragment of claim 57, wherein the medicament manufactured is used to inhibit angiogenesis.

59. The use of a therapeutically effective fragment of claim 57, wherein the medicament manufactured is used to inhibit endothelial cell growth.

60. A method of inhibiting radiation induced injury, comprising contacting cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

61. A method of inhibiting chemotherapy induced injury, comprising contacting cells with a pharmaceutical composition comprising at least one protein selected from the group consisting of:

(a) therapeutically effective fragments of calreticulin;

(b) therapeutically effective variants of calreticulin; and

(c) calreticulin.

62. The use of therapeutically effective fragments of calreticulin, variants of calreticulin, or calreticulin in the manufacture of a medicament to treat or prevent radiation induced injury or chemotherapeutic induced injury.

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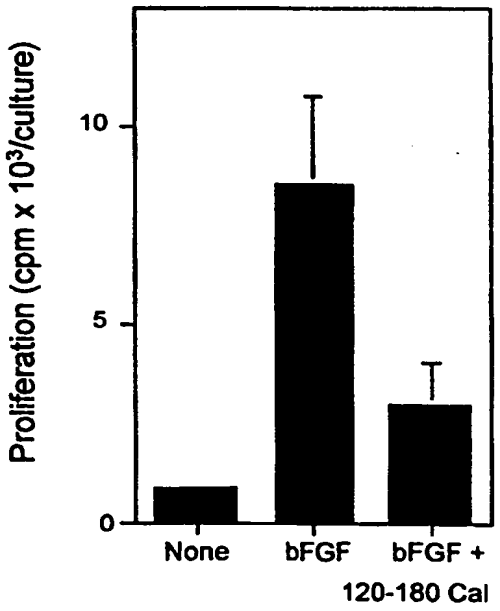
(54) Title: USE OF CALRETICULIN AND CALRETICULIN FRAGMENTS TO INHIBIT ENDOTHELIAL CELL GROWTH AND ANGIOGENESIS, AND SUPPRESS TUMOR GROWTH

(57) Abstract

Methods for inhibiting endothelial cell growth and angiogenesis, and suppressing tumor growth using calreticulin, fragments of calreticulin and variants of calreticulin are provided. Such methods are useful for the treatment of cancer and diseases associated with unwanted angiogenesis, for example chronic retinal detachment.



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INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 99/23240

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 36643 A (UNIVERSITY OF ALBERTA) 21 November 1996 (1996-11-21) the whole document ---	1-9
X	WO 96 23001 A (S DEDHAR & R ST-ARNAUD) 1 August 1996 (1996-08-01) the whole document ---	1-10
X	WO 95 13828 A (NEY YORK UNIVERSITY & GENERAL HOSPITAL CORPORATION) 26 May 1995 (1995-05-26) the whole document & US 5 591 716 A cited in the application --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 426 097 A (D M STERN ET AL.) 20 June 1995 (1995-06-20) cited in the application the whole document ---	1-10
X	CA 2 140 814 A (S DEDHAR) 24 July 1996 (1996-07-24) the whole document ---	1-10
X	File Medline, abstract no. 1998073667, 1998 XP002133012 & E DAI ET AL.: "Calreticulin, a potential vascular regulatory protein, reduces intimal hyperplasia after arterial injury" ARTERIOSCLEROSIS, THROMBOSIS AND VASCULAR BIOLOGY, vol. 17, no. 11, November 1997 (1997-11), pages 2359-2368, abstract ---	1-10
P,X	CHEMICAL ABSTRACTS, vol. 130, no. 14, 5 April 1999 (1999-04-05) Columbus, Ohio, US; abstract no. 177891, S E PIKE ET AL.: "Vasostatin, a calreticulin fragment, inhibits angiogenesis and suppresses tumor growth" XP002133015 & JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 12, 1998, pages 2349-2356, TOKYO, JP ISSN: 0022-1007 abstract ---	1-50,56
P,X	S E PIKE ET AL.: "Calreticulin and calreticulin fragments are endothelial cell inhibitors that suppress tumor growth" BLOOD, vol. 94, no. 7, 1 October 1999 (1999-10-01), pages 2461-2468, XP002133011 new york the whole document --- -/--	1-50,56

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	File Medline, abstract 97218114, 1997 XP002133013 & U KISHORE ET AL.: "Release of calreticulin from neutrophils may alter C1q-mediated immune function" BIOCHEMICAL JOURNAL, vol. 322, no. 2, 1 March 1997 (1997-03-01), pages 543-550, abstract ---	31
X	File Medline, abstract 93185299, 1993 XP002133014 & J G ROUTSIAS ET AL.: "Calreticulin synthetic peptide analogues; anti-peptide antibodies in autoimmune rheumatic diseases " CLINICAL AND EXPERIMENTAL IMMUNOLOGY, vol. 91, no. 3, March 1993 (1993-03), pages 437-441, abstract -----	31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/ 23240

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-30 and 56 (at least partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/ composition.

2. [X] Claims Nos.: 51-55
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 51-55

As the products defined in the above claims are not characterized by any other feature that their hypothetical ability to bind to a solid phase where peptides according to claim 35 are immobilized, with no example provided, a search could not be conducted for lack of clarity (Art. 6 and Rule 6 PCT).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. ...nal Application No
PCT/US 99/23240

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9636643 A	21-11-1996	AU 5512096 A	29-11-1996
WO 9623001 A	01-08-1996	US 5854202 A	29-12-1998
		AU 3920395 A	14-08-1996
		WO 9905172 A	04-02-1999
		EP 0807121 A	19-11-1997
WO 9513828 A	26-05-1995	US 5591716 A	07-01-1997
		AU 1051695 A	06-06-1995
US 5426097 A	20-06-1995	NONE	
CA 2140814 A	24-07-1996	NONE	

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
...T/US 99/23240

A. CLASSIFICATION OF SUBJEC... ...TER
IPC 7 C12N15/11 ...07K14/47 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 36643 A (UNIVERSITY OF ALBERTA) 21 November 1996 (1996-11-21) the whole document ---	1-9
X	WO 96 23001 A (S DEDHAR & R ST-ARNAUD) 1 August 1996 (1996-08-01) the whole document ---	1-10
X	WO 95 13828 A (NEY YORK UNIVERSITY & GENERAL HOSPITAL CORPORATION) 26 May 1995 (1995-05-26) the whole document & US 5 591 716 A cited in the application --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2000	28/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Masturzo, P

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 426 097 A (D M STERN ET AL.) 20 June 1995 (1995-06-20) cited in the application the whole document	1-10
X	CA 2 140 814 A (S DEDHAR) 24 July 1996 (1996-07-24) the whole document	1-10
X	File Medline, abstract no. 1998073667, 1998 XP002133012 & E DAI ET AL.: "Calreticulin, a potential vascular regulatory protein, reduces intimal hyperplasia after arterial injury" ARTERIOSCLEROSIS, THROMBOSIS AND VASCULAR BIOLOGY, vol. 17, no. 11, November 1997 (1997-11), pages 2359-2368, abstract	1-10
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P,X	S E PIKE ET AL.: "Calreticulin and calreticulin fragments are endothelial cell inhibitors that suppress tumor growth" BLOOD, vol. 94, no. 7, 1 October 1999 (1999-10-01), pages 2461-2468, XP002133011 new york the whole document	1-50,56

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 99/23240

C.(Continuation) DOCUMENTS C[redacted]ERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

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PCT/US 99/ 23240

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4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 51-55

As the products defined in the above claims are not characterized by any other feature that their hypothetical ability to bind to a solid phase where peptides according to claim 35 are immobilized, with no example provided, a search could not be conducted for lack of clarity (Art. 6 and Rule 6 PCT).

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. ...nal Application No
.../US 99/23240

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		AU 3920395 A	14-08-1996
		WO 9905172 A	04-02-1999
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WO 9513828 A	26-05-1995	US 5591716 A	07-01-1997
		AU 1051695 A	06-06-1995
US 5426097 A	20-06-1995	NONE	
CA 2140814 A	24-07-1996	NONE	